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RESEARCH ARTICLE

Synthesis and Biological Evaluation of Some New Chalcone Derivatives as Anti-inflammatory Agents

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ARTICLE HISTORY

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Abstract: ***Aim*:** The present research work aims to prepare a series of 1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy)phenyl)-3-phenylprop-2-en-1-one derivatives.

***Methods*:** The major compound was achieved by the reaction of indole with chloroacetylchloride in benzene afforded 2-chloro-1-(indoline-1-yl) ethanone which reacts o- hydroxy acetophenone in presence of acetonitrile to form 2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one then goes through aldol condensation to give various final derivatives.

***Results and Conclusion*:** After the synthesis of compounds, the synthesized compounds were characterized by checking their solubility, melting point, thin layer chromatography, IR, 1HNMR spectral data and elemental analysis. All of the prepared derivatives were evaluated for their anti-inflammatory activity on wistar albino rats by following the carrageenan-induced Rat Hind Paw Edema model.

Keywords: Chalcone derivatives, anti- inflammatory activity, carrageenan model, substituted benzaldehyd chalcone derivatives, substituted benzaldehyde, thin layer chromatography.

1. INTRODUCTION

Medicinal chemistry is demarcated as a reliant advanced science that is comprised of applied and basic science. It includes the discovery, development, identification, and interpretation of the mechanism of action of medicinally active ingredients at the molecular level. Medicinal chemistry can be said as the hub of synthetic & applied chemistry and cellular pharmacology which helps to underline the study of SAR of drug molecules. At an institutional level in the Us S., medicinal chemistry was first served as part of pharmaceutical chemistry (1909- 1920), which was then modified and named the division of medicinal chemistry, by the American Chemical Society [1]. Medicinal Chemistry especially works on a detailed study of the structure of molecules and the target of the molecules which are present in the host body [2, 3].

The term "chalcone" is a generic term used to describe compounds with the 1,3-diphenylprop-2-en-1-one framework (Fig. **1**). Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Chemically these are 1,3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three-carbon α,β-unsaturated carbonyl system [4].

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1.1. Inflammation

Inflammation is the body's extreme response to any form of injury. Pain, redness, heat or warmth, and swelling are the four major symptoms of inflammation. The arterioles in the surrounding tissue dilate when a part of the human body is injured. This results in increased blood flow to the area (redness). Vasoactive substances also enhance the permeability (pore size) of these arterioles, allowing blood cells, chemicals, blood proteins, and fluid to collect in that area. This fluid build-up produces swelling and can cause discomfort by compressing nerves in the area. Furthermore, prostaglandins may cause nerve irritation and hence contribute to pain. The presence of prostaglandins (PGs) and kinins in inflammatory exudates suggest that they have a function in inflammation. COX-2 has a definite link to inflammation [5]. Inflammation is a multifactorial process that reflects the organism's response to diverse stimuli and is linked to a number of ailments such as arthritis, asthama, and psoriasis all of which necessitate long-term or recurring therapy. The inflammatory response is a broad response to infection and tissue injury that aims to clear cellular debris, pinpoint invading organisms, and stop infections from spreading. The symptoms of an inflammatory reaction include reddening of the localised area, swelling, discomfort, and an increase in temperature.

1.1.1. Inflammation and their Types

Various types of inflammations and their adverse effects are shown in Fig. (**1**) with the caption of inflammation and its types.

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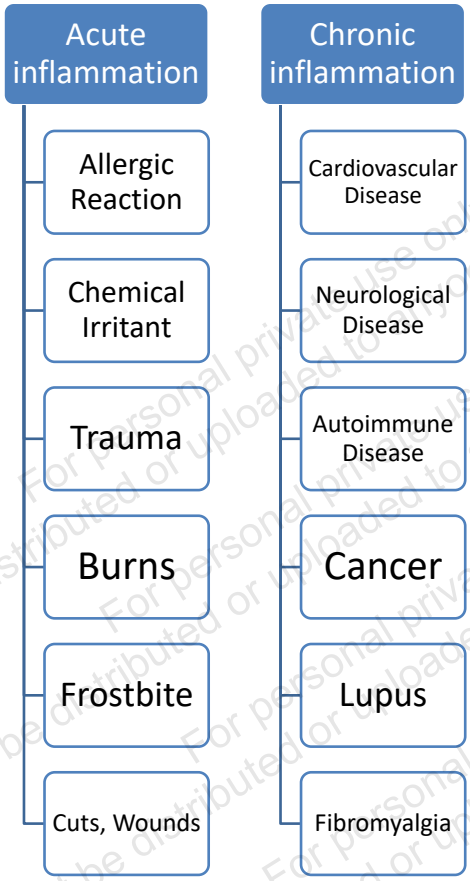


Fig. (1). Inflammation and their types.

1.1.1.1. Chronic Inflammation

Chronic Inflammation has a long duration of action and tissues are destroyed severely. Majorly lymphocytes are the inflammatory cells, which activate fibroblasts and lead to the putting down of fibrosis and collagen. If the body's defense system is incapable to remove the infection, it attempts to scrap it off which restricts the formation of nodules named granulomas, granulomas are a bunch of self-protective sacs. Example- Tuberculosis which becomes chronic and leads to the formation of granuloma [6].

1.1.1.2 Acute Inflammation

The inflammation has a shorter duration of action like days to some weeks and ranges from mild to severe. This acute inflammatory response is described as a collection of overlapping events, increased blood flow, accumulation of tissue fluid, migration of leukocytes, increased core temperature, pain, and suppuration.

1.1.1.3. Inflammation and their Causes

There are so many causes of inflammation and some of them are listed below:-

- Microorganisms like bacteria, fungi, viruses, protozoa,
- Physical causes- temperature, stony, mechanical injury, ultraviolet and ionizing radiation.
- Organic Chemical agent- microbial poisons, organic toxic materials
- Inorganic Chemical agent- acids, alkalis.

1.1.1.4. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs have become an important aspect of the pharmacological treatment of pain (at low doses) and inflammation since the extraction of salicin from the folk medicine willow bark in 1829. (at higher doses). NSAIDs are popular because, unlike opioids, they do not cause drowsiness or respiratory depression and have a low likelihood of addiction. Certain NSAIDs, such as ibuprofen and aspirin, have gained a reputation for being generally safe and are now accessible without a prescription over-the-counter. The major enzyme in the manufacture of prostaglandins (PGs), the principal mediators of inflammation, discomfort, and increased body temperature, is cyclooxygenases (COX) or prostaglandin endoperoxide synthases (PGHS) (hyperpyrexia). Arachidonic acid, the precursor of prostaglandins, is used to make them.

1.1.1.5. Mechanism of Action of NSAIDs

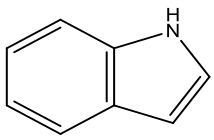
NSAIDs act by inhibiting the action of cyclooxygenase enzymes (COX-1 or COX-2). All of the NSAIDs appear to share at least one common mechanism, namely inhibition of COX enzyme which leads to a decrease in the synthesis of various prostaglandins (PGs) and thromboxanes (TXs).

It was noted that the inhibition of aspirin causes prostaglandin (PG) synthesis. Around 1972, the mechanism of action of NSAIDs was proposed that it works by inhibiting the COX enzymes or PG G/H synthase and subsequent PG formation [7]. It was revealed that their therapeutic benefits and toxicity are related to their ability to inhibit COX.

1.2. Indole

There are several significant moieties, including indole, which is one of the most important. Indole is a very essential aromatic heterocyclic molecule. In 1866, Adolf von Baeyer produced indole by reducing oxindole in the presence of a catalytic quantity of zinc dust, and in 1869, he devised its formula. Indole has a phenyl ring attached to a pyrrole ring. It has a molecular formula C_8H_7N and the molecular weight is 117.15g/mol. It is a white, feces-like odour compound and shows solubility in hot water and organic solvents such as ethanol, acetone, and benzene [8, 9].

Indole and its derivatives are under the active class of compounds with a wide range of biological activities including anti-inflammatory *e.g.* Indomethacin [10], anti-bacterial *e.g.* Indolmycin [11], antipsychotic *e.g.* Oxypertine [12], sexual disorder *e.g.* Yohimbine [13], Antihypertensive *e.g.* Pindolol [14], anti-HIV *e.g.* Delavirdine [15] anticancer *e.g.* Ellipticine [16], serotonin receptor agonists *e.g.* Sumatriptan [17], HMG-CoA reductase inhibitors *e.g.* Fluvastatin [18], serotonin 5-HT3 receptor antagonists *e.g.* Ondansetron and phosphodiesterase-5 (PDE5) inhibitors *e.g.* Tadalafil [19]. The structure of indole is represented in Fig. (**2**).



Indole

Fig. (2). Structure of Indole.

2. EXPERIMENTAL

2.2. Materials & Methods

2.2.1. Synthetic Procedure

Scheme **1** represents the synthesis of indole (**1**) derivatives and the reaction scheme is comprised of the 3 steps: Preparation of compound **2** is described in Scheme **2**. Preparation of 2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one (Compound **3**) is shown in Scheme **3**. Scheme **4** tells about the general procedure for the preparation of the series of 1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy)phenyl)-3-phenylprop-2-en-1-one (4a-4d).

Step 1- Synthesis of Compound 2 (2-Chloro-1-(1H-indol-1-yl)ethan-1-one)

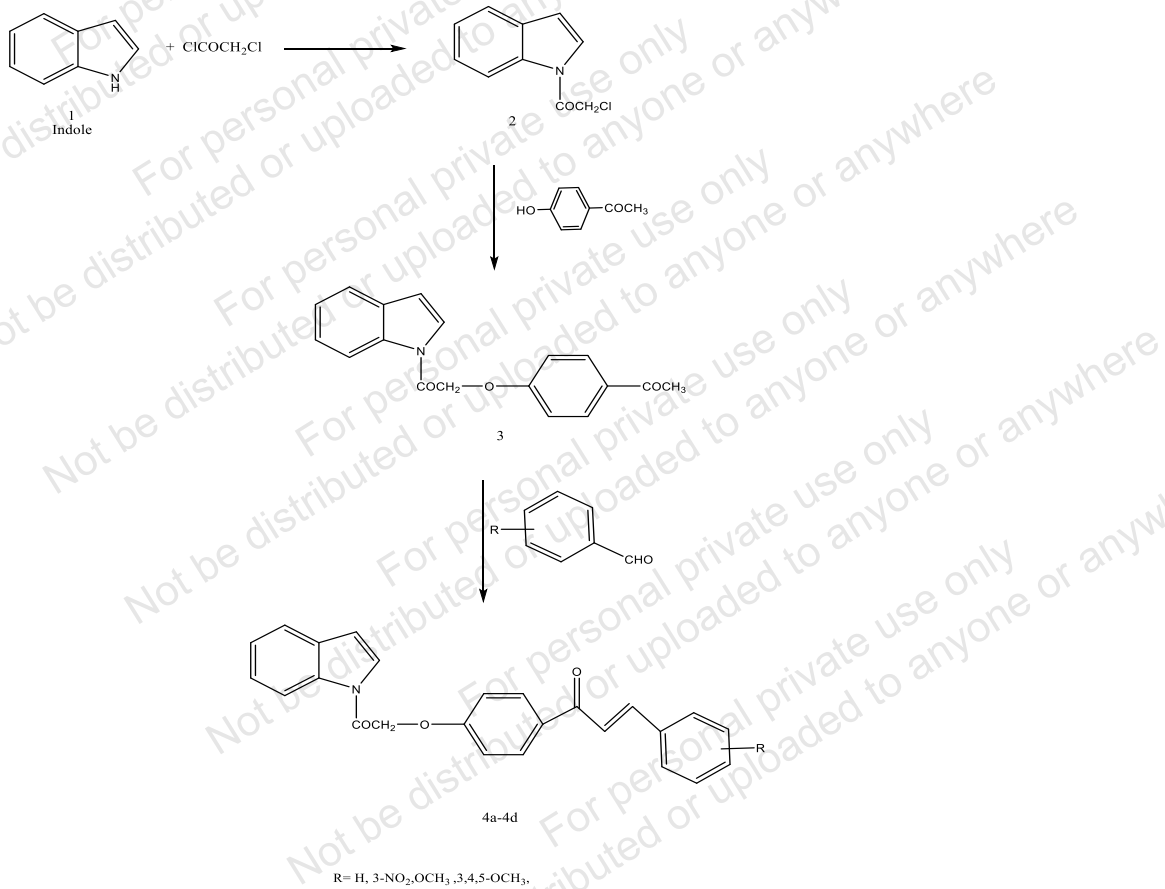
Procedure- In a round bottom flask a solution of Indole (0.02 mol) in dry benzene (100 ml) is taken and the mixture is heated under reflux. In this refluxing solution, chloroacetyl chloride (0.01 mol) was added slowly. After this the reaction mixture is refluxed for 4 hrs. The precipitate is filtered, dried and recrystallized with ethanol to get crystals of 2-chloro-1-(1H-indol-1-yl) ethanone. After this, melting point and TLC was observed [20, 21] (C. A. Winter *et al.,* 1962) (Mutschler *et al.*, 1978).

Step 2- Synthesis of Compound 3 (2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one)

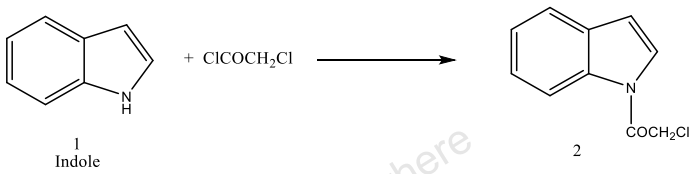
Compound **2** (2-chloro-1-(1H-indol-1-yl) ethenone) (0.01 mol) dissolved in acetonitrile (40 ml) while stirring, after that *p*-hydroxy acetophenone (0.01 mol) dissolved in acetonitrile (10ml) with (0.02mole) of anhydrous K_2CO_3 is taken in a round bottom flask. The reaction mixture is heated under reflux for 6 h. The reaction mixture is cooled and the solvent is evaporated under reduced pressure. After that solid is separated, washed with water and dried at normal room temperature. TLC and melting point were observed.

Step 3- General Procedure for the Synthesis of Compound 4a-4d (1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy) Phenyl)-3-phenylprop-2-en-1-one)

Procedure-Compound **3** (2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one) (0.002 mol, was taken in a round bottom flask and 20ml of acetonitrile was added to it. In this solution, anhydrous K_2CO_3 (0.004 mol) was added. After that substitutedbenzaldehydes (0.002 mol) were added to this solution. Then the reaction mixture is refluxed for 7 hours at 20-25°C. The reaction mixture is cooled and the



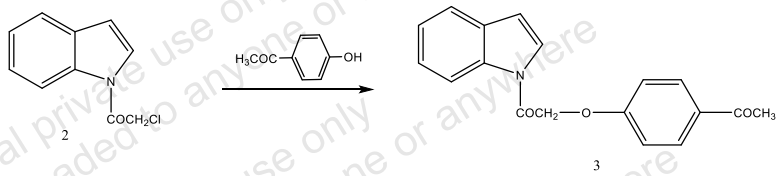
Scheme **1**. Synthesis of indole derivatives.



1
Indole

2

Scheme **2**. Synthesis of 2-chloro-1-(1H-indol-1-yl)ethan-1-one.



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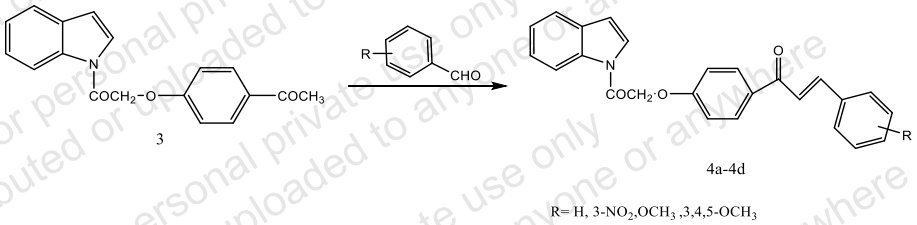
3

Scheme **3**. Synthesis of 2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one.



3

4a-4d

R= H, 3-NO_2,OCH_3 ,3,4,5-OCH_3

Scheme **4**. General procedure for the synthesis of derivatives (**4a-4d**).

solvent is evaporated under reduced pressure. After that solid is separated, washed with water, and dried at normal room temperature. Melting point and TLC were observed [22].

2.2.2. Characterization of Synthesized Compounds

The synthesized compounds will be subjected to characterization using the following techniques.

A. Physicochemical characterization as Melting point and TLC

B. Spectral methods

a) IR-Spectroscopy

b) ^{1}H NMR-Spectroscopy

2.2.2.1. Melting Point Determination

The melting points of the synthesized compounds as well as intermediates were determined by open capillary methods and were uncorrected.

2.2.2.2. Thin Layer Chromatography (TLC)

The purity of the compounds was routinely checked by thin-layer chromatography (TLC) using iodine vapours for the detection of spots.

2.2.2.3. Infra-red Spectroscopy

The IR spectra of the synthesized compounds were recorded in potassium bromide discs and on FT-IR spectrophotometer at FTIR lab SAIF, Punjab University, Chandigarh.

2.2.2.4. Nuclear Magnetic Resonance Spectroscopy

The ^{1}H-NMR spectra of the synthesized compounds were recorded in DMSO (dimethylsulphoxide) using BROKE ADVANCE NEO at 500MHz spectrophotometer, at Punjab University, Chandigarh.

2.3. Pharmacological Evaluation

2.3.1. Experimental Animals

Male wistar albino rats (150-200 g) were obtained from Institutional Animal House, IFTM University, Lodhipur-Rajput, Moradabad, India were used for analgesic and anti-inflammatory activity. The rats were held in reserve in polypropylene cages at 30±2 °C, relative humidity of 55-65%. All the rats were acclimatized to laboratory situations for a week previous to start the experiment. All the rats were fed with standard animal feed and water *ad libitum*. The study protocol was reviewed and approved by Animal Ethical Committee, Committee for Control and Supervision of Experiments on Animals (CPCSEA) Govt. of India, New Delhi.

2.3.2. Acute Toxicity Study

Acute toxicity study was done to determine the dose of the derivatives and in this study, a software is used to calculate the dosage.

Acute toxicity study was done by using software TEST (toxicity estimation software tool). All the structures were prepared in the software and toxicity value was obtained by concensus method to predict oral rat LD50 (mg/kg). The oral- LD50 dose of prepared derivatives is shown in Table **1**.

As per the data obtained from the toxicity study, an average dose 100mg/kg (followed by 1/10 of the dose obtained) was taken for the testing of derivatives.

Table 1. Acute toxicity study (Oral Rat LD50).

Compound Code	Oral LD50(mg/kg)
4a	1183.21
4b	1698.51
4c	1184.28
4d	1151.29

2.3.3. Evaluation of Anti-Inflammatory Activity

2.3.3.1. Carrageenan-Induced Rat Paw Edema Model

The wistar albino rats were divided into four groups containing six rats (one control, one standard & two test groups) and acute inflammation was induced according to edema assay. The extract was evaluated for the anti-inflammatory activity. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% Carrageenan in normal saline in the right hind paw of the rats, 1 h after the administration of the drug/extract. The paw diameter was measured by using digital calipers at the intervals of 1, 2, and 3 h after the Carrageenan injection. Indomethacin (10 mg/kg, orally) was used as a standard drug [23, 24].

Control Group: 1% Carrageenan solution (5 ml/kg b.w).

Standard Group: Carrageenan + Indomethacin (10 mg/kg b.w).

Test Groups: Carrageenan + test compounds (100 mg/kg b.w).

The anti-inflammatory activity was calculated as percentage inhibition of Carrageenan induced paw edema using the following formula.

% inhibition= (1-Vt/Vc)x 100

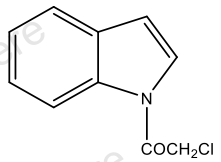
Where: Vt = paw diameter in treated; Vc = paw diameter in control.

2.4. Chemistry

The synthesis of chalcone derivatives was prepared by chloroacetylation of indole and then reacting the resulting product with hydroxy acetophenone and finally, derivatives were prepared by refluxing substituted benzaldehyde with the 2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one by following aldol condensation method. In this study, like anti-inflammatory drugs, some new chalcone derivatives were devised and produced. Physicochemical characterization of the compounds is described in Table **2**. Further Table **3** contains the anti- inflammatory response of the prepared derivatives.

2.4.1. Spectral Data of Compounds

Compound 2

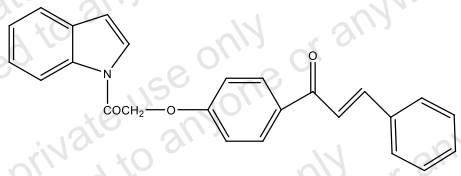


2-chloro-1-(1H-indol-1-yl)ethan-1-one

IR (KBr,cm^{-1}): 3549.91 (O-H str), 3414.77 (C=C str), 1620.21 (C=O str), 1451.68 (C=C str), 1349.72 (NO_2 str), 1201.66 (C-H bend), 747.33 (monosubstituted Ar-R), 619.109 (C-Cl str).

1 H-NMR (DMSO) δ; 0.52 (s, 3H, C=O-CH), δ; 1.42 (M, 5H, CHO) δ; 0.29 (s, 1H, XCH), δ; (m, 8H, Ar-H), δ;1.0 (d, 2H, C-CH), δ; 1.90 (d, 4H, C-CH), δ; 2.47 (d, 8H, XCH), δ; 7.37 (d, 8H, C=CH), 7.64 (t, 9H, Ar-OH), δ; 8.29 (m, 9H, Ar-H), δ; 2.60 (d, 8H, C=C-CH), δ; 2.17 (s, 1H, Ar-CH), δ; 1.43 (s, 1H, O-CH), δ; 1.39 (s, 1H, C=CH), δ; 3.75 (m, 14H, C=C-H).

Compound 4-a



(E)-1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy)phenyl)-3-phenylprop-2-en-1-one

IR (KBr, cm^{-1}): 3415.72 (N-H str), 2074.01 (C=C str), 1619.15 (C=O str), 1511.92 (C=C str Ar.), 1485.75 (C=C str

Table 2. Physicochemical characterization of compounds.

Compound Code	Molecular Formula	Molecular Weight	Solubility	Melting Point(°C)	Colour	Rf Value*
1	C_8H_7N	117.14	Acetone, Ethyl acetate	57°C	Dark brown	0.47
2	$C_{10}H_8CINO$	193.63	Acetone, Chloroform, Ethanol	76°C	Reddish brown	0.65
3	$C_{18}H_{15}NO_3$	293.32	Acetone, Ethyl acetate	74°C	Dark brown	0.84
4a	$C_{25}H_{19}NO_3$	381.43	DMSO, Chloroform	121°C	Greyish black	0.66
4b	$C_{25}H_{18}N_2O_5$	426.43	DMSO, Chloroform	139°C	Dark brown	0.81
4c	$C_{26}H_{21}NO_4$	411.46	DMSO, Chloroform	125°C	Greyish black	0.75
4d	$C_{27}H_{23}NO_5$	441.48	DMSO, Ethyl acetate	136°C	Greyish black	0.83

Note: * Mobile phase ethyl acetate and n- hexane (1:2).

Table 3. Anti-inflammatory activity of synthesized compounds.

Treatment	Dose	Mean Change in Paw Volume			% Inhibition		
		60 m	120 m	180 m	60 m	120 m	180 m
Control	5 ml/kg	0.526±0.14	0.583±0.17	0.641±0.19	----	----	----
Indomethacin	10	0.129±0.10*	0.163±0.09*	0.171±0.06*	69.77	72.04	73.32
4a	100	0.238±0.18*	0.212±0.08*	0.297±0.09*	54.85	63.63	53.66
4b	100	0.289±0.10*	0.249±0.09*	0.328±0.08*	45.05	57.28	48.83
4c	100	0.257±0.10*	0.235±0.04*	0.343±0.09*	51.14	59.69	46.48
4d	100	0.263±0.03*	0.259±0.05*	0.216±0.09*	50.00	55.57	66.30

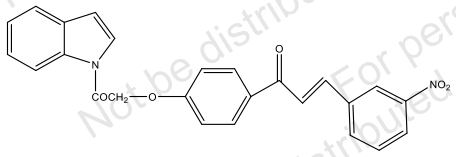
Note: Dose of compound = 100 mg/kg; indomethacin dose = 10 mg/kg,

*Results are mean ± S.E.M. (n=6) *P<0.05.

Ar), 1451.14 (C=C str Ar.), 1153.75 (C-H bend), 836 (C-H bend aromatic, disubstituted), 611 (monosubstituted Ar), 748 (C-H bend Ar).

1 H-NMR (DMSO) δ; 0.61 (s,1H, -(C=O) R, δ; 2.00 (s, 1H, C-C-H), δ; 1.00 (s,1H,C-C-H), δ; 1.72 (d, 2H,C=C-C-H), δ;4.69(d, 2H,C=CH), δ; 2.50 (d, 6H,C=C-C-H), δ;5.76 (m, 8H, N-H-C-O), δ;4.95 (t,3H,C=CH), δ;2.69 (m, 5H,Ar-C-H), δ; 1.17 (d, 2H ,C-C-H), δ;6.82 (m, 5H,Ar-H), δ;3.59 (s,1H,O-C-H).

Compound 4-b

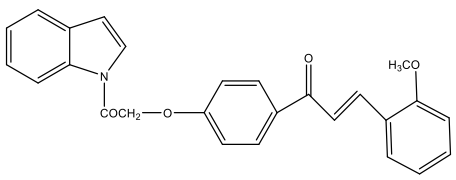


(E) -1- (4 -(2-(1H –indol –1-yl)–2-oxoethoxy) phenyl) –3 -(3-nitrophenyl)prop-2-en-1-one

IR (KBr, cm^{-1}) : 3469.40 (N-H str), 3415.56 (N-H str), 2032 (C=C str Alkyne), 1620 (C=C str Aromatic), 1528 (C=O str), 1347 (C=C str Aromatic), 745 (C-H monosubstrate Ar-R), 619 (=C-H Alkene).

1 H-NMR (DMSO) δ ; 2.32 (s, 1H, C=C-CH), δ ; 1.00 (s,1H, C-CH), δ ; 1.25 (s, 1H, C-CH), δ ; 1.98 (d, 2H, C-CH) δ ; 2.13 (9d, 2H, C=CH), δ ; 1.55(t, 3H, C=C-CH), δ ; 2.74(d, 2H, Ar-CH),δ;2.80 (d, 2H, Ar-CH), δ ; 3.23 (d, 2H, O-CH), δ ; 3.87 (d, 2H, O-CH), δ ; 2.09 (s, 1H,C-CH), δ ; 3.23 (s, 1H, O-CH), δ ; 7.96 (d, 4H, Ar - OH), δ ; 5.67 (d, 2H, C=CH), δ ; 7.10 (d, 2H, Ar - H), δ ; 8.48 (d, 2H, C=CH), δ ; 5.44 (m, 5H, O - CH), δ ; 2.43 (d, 2H, C=C-CH), δ ; 6.65 (s, 1H, Ar - H).

Compound 4-c



(E)-1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy)phenyl)-3-(2-methoxyphenyl)prop-2-en-1-one

IR (KBr, cm^{-1}) : 3424.13 (N-H str), 1651.64(C=O str), 1369 (C=C str Ar), 1269.97(C=O str), 847.38 (di-substituted para), 747 (C-H bend Aromatic), 609 (C-H bend Ar).

1 H-NMR (DMSO) δ ; 4.17 (s, 1H, Ar - O-H), δ ; 1.94(s, 1H, C-CH), δ ; 2.49(m, 4H, C=C-CH), δ ; 2.72 (d, 2H, Ar - CH), δ ; 3.09 (d, 2H, Ar - CH), δ ; 6.31 (s, 1H, C=C- CH), δ ; 6.45 (s, 1H, C=C-CH).

CONCLUSION

The synthesis of chalcone derivatives was prepared by chloroacetylation of indole and then reacting the resulting product with hydroxy acetophenone and finally derivatives were prepared by refluxing substituted benzaldehyde with the 2-(4-acetylphenoxy)-1-(1H-indol-1-yl)ethan-1-one. The inhibition of swelling in carrageenan induced edema in rat paws brought about by oral administration of the drug is shown in Table **3**. All the synthesized compounds tested for anti-inflammatory activity showed inhibition of edema ranging from 50.00 to 66.3%, especially compounds **4a** and **4d** which demonstrated very good anti-inflammatory activity. In particular, 1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy)phenyl)-3-phenylprop-2-en-1-one (4a) and (E)-1-(4-(2-(1H-indol-1-yl)-2-oxoethoxy)phenyl)-3-(2-methoxyphenyl)prop-2-en-1-one (4c) were equipotent with standard drug indomethacin (92.7%) in inhibiting paw edema.

However, the current study of the prepared derivatives represents their biological activity against inflammation but is not as good as the standard drug indomethacin. It can conclude that the derivatives are much near to standard drugs and the aim of this study was to do research at the institutional level.

LIST OF ABBREVIATIONS

Ar = Aromatic
bend = Bending

d	=	Doublet
DMSO	=	Dimethyl Sulfoxide
g	=	Gram
m	=	Multiplet
s	=	Singlet
str	=	Stretching

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was reviewed and approved by Animal Ethical Committee, Committee for Control and Supervision of Experiments on Animals (CPCSEA) Govt. of India, New Delhi.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the reported experiments were in accordance with The US National Research Council's "Guide for the Care and Use of Laboratory Animals".

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

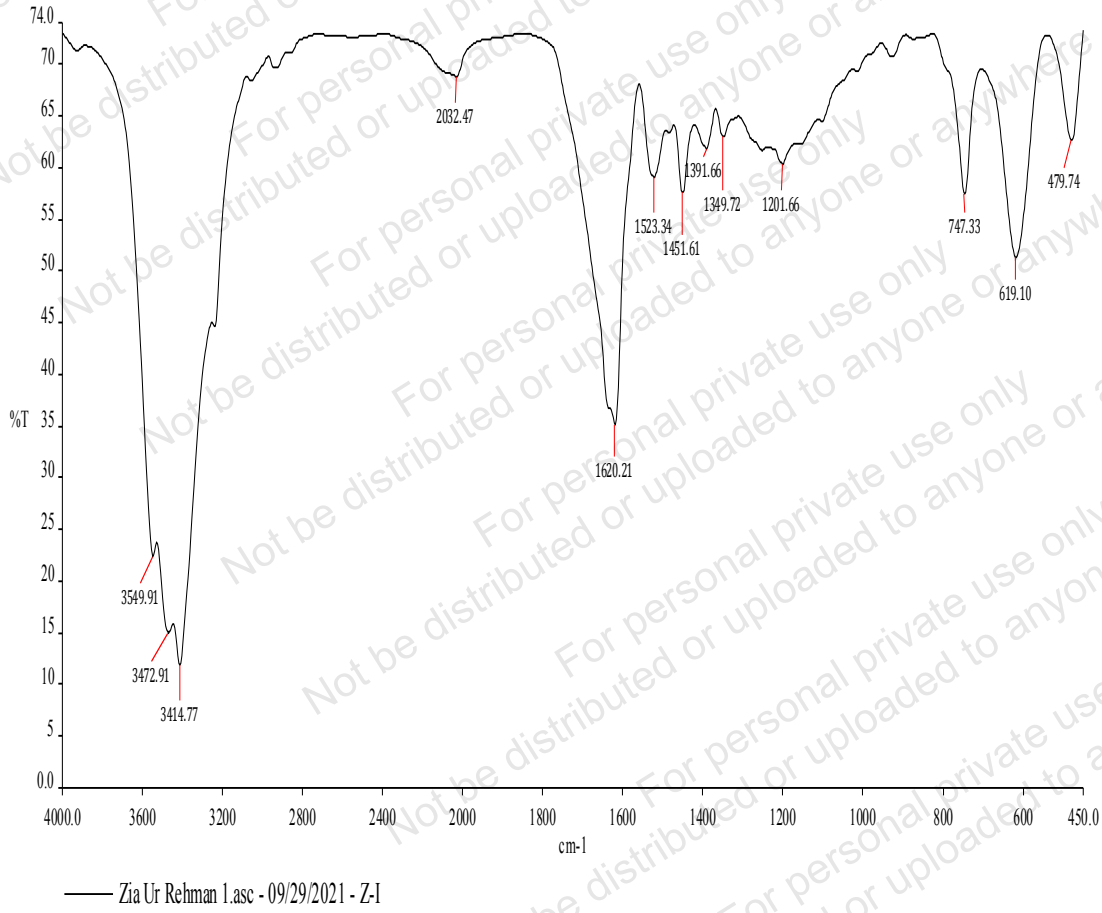
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

APPENDIX

IR Spectra of Compound 4a



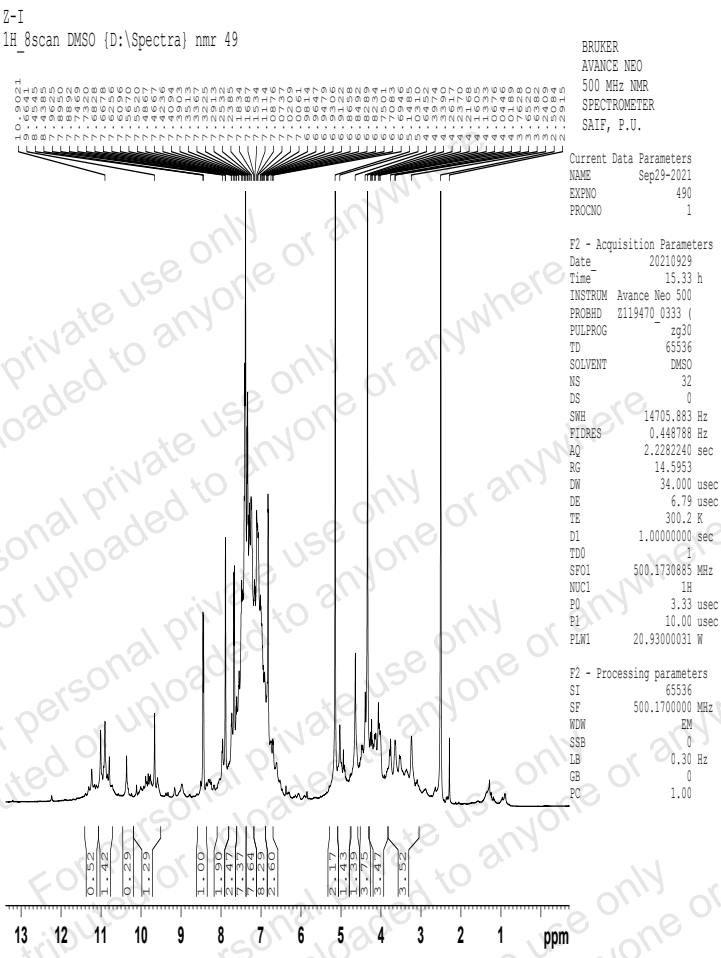
1H-NMR

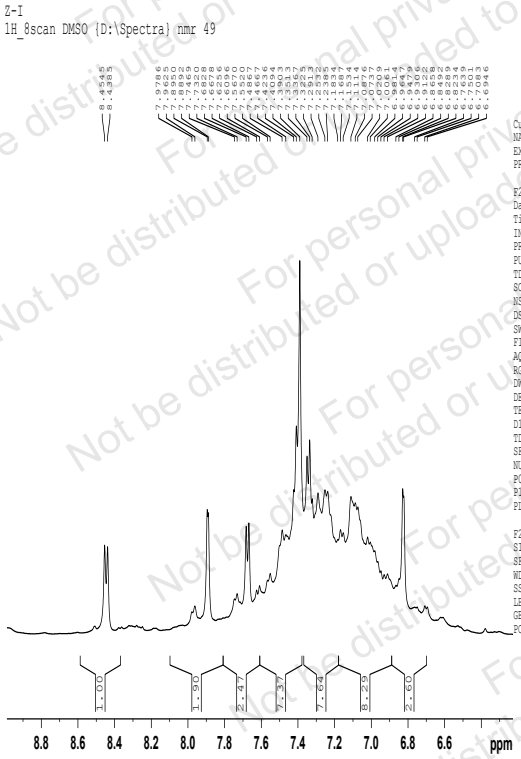

```
BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

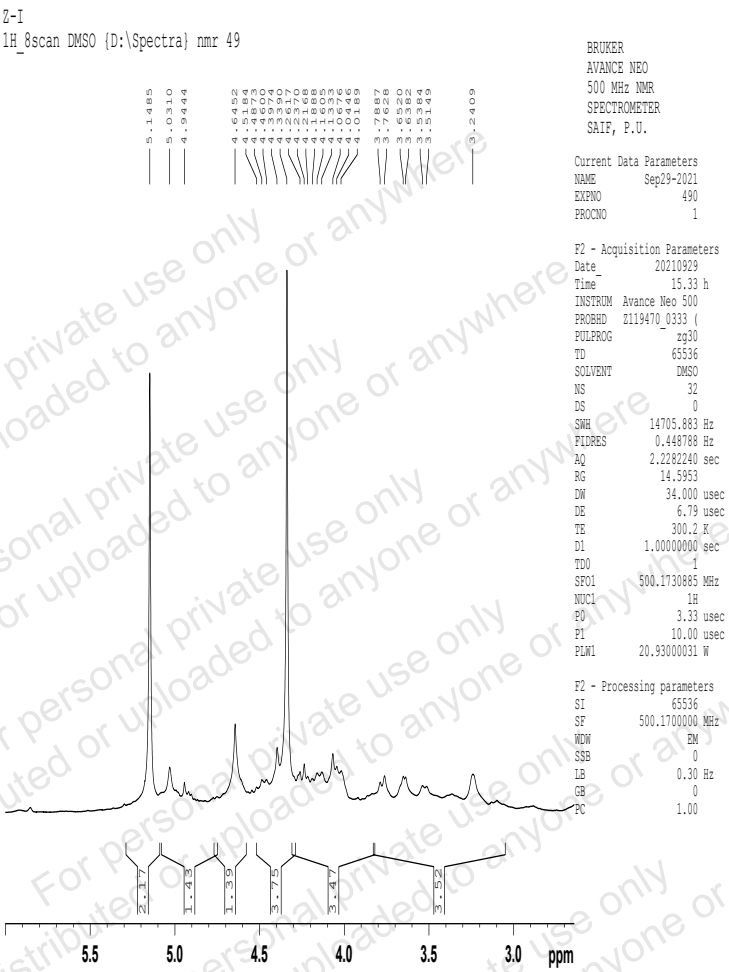
Current Data Parameters
NAME         Sep29-2021
EXPNO               490
PROCNO                1

F2 - Acquisition Parameters
Date_          20210929
Time             15.33 h
INSTRUM  Avance Neo 500
PROBHD   Z119470_0333 (
PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                    32
DS                     0
SWH           14705.883 Hz
FIDRES         0.448788 Hz
AQ             2.2282240 sec
RG               14.5953
DW                34.000 usec
DE                 6.79 usec
TE                300.2 K
D1           1.00000000 sec
TD0                    1
SFO1       500.1730885 MHz
NUC1                  1H
P0                 3.33 usec
P1                10.00 usec
PLW1        20.93000031 W

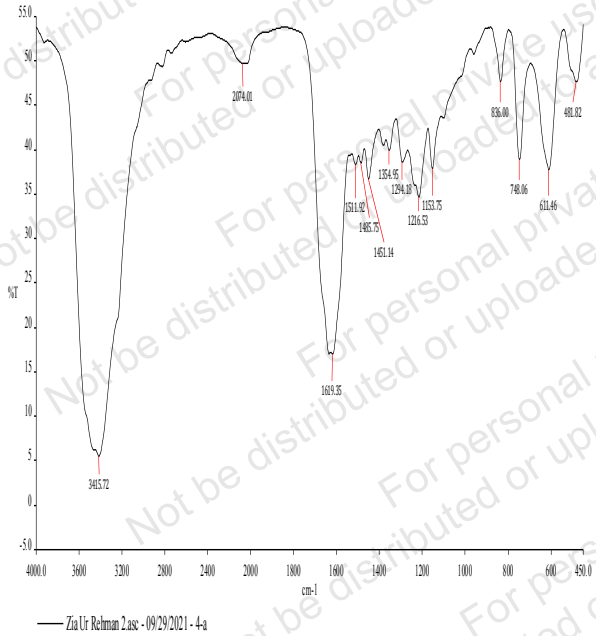
F2 - Processing parameters
SI                 65536
SF            500.1700000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



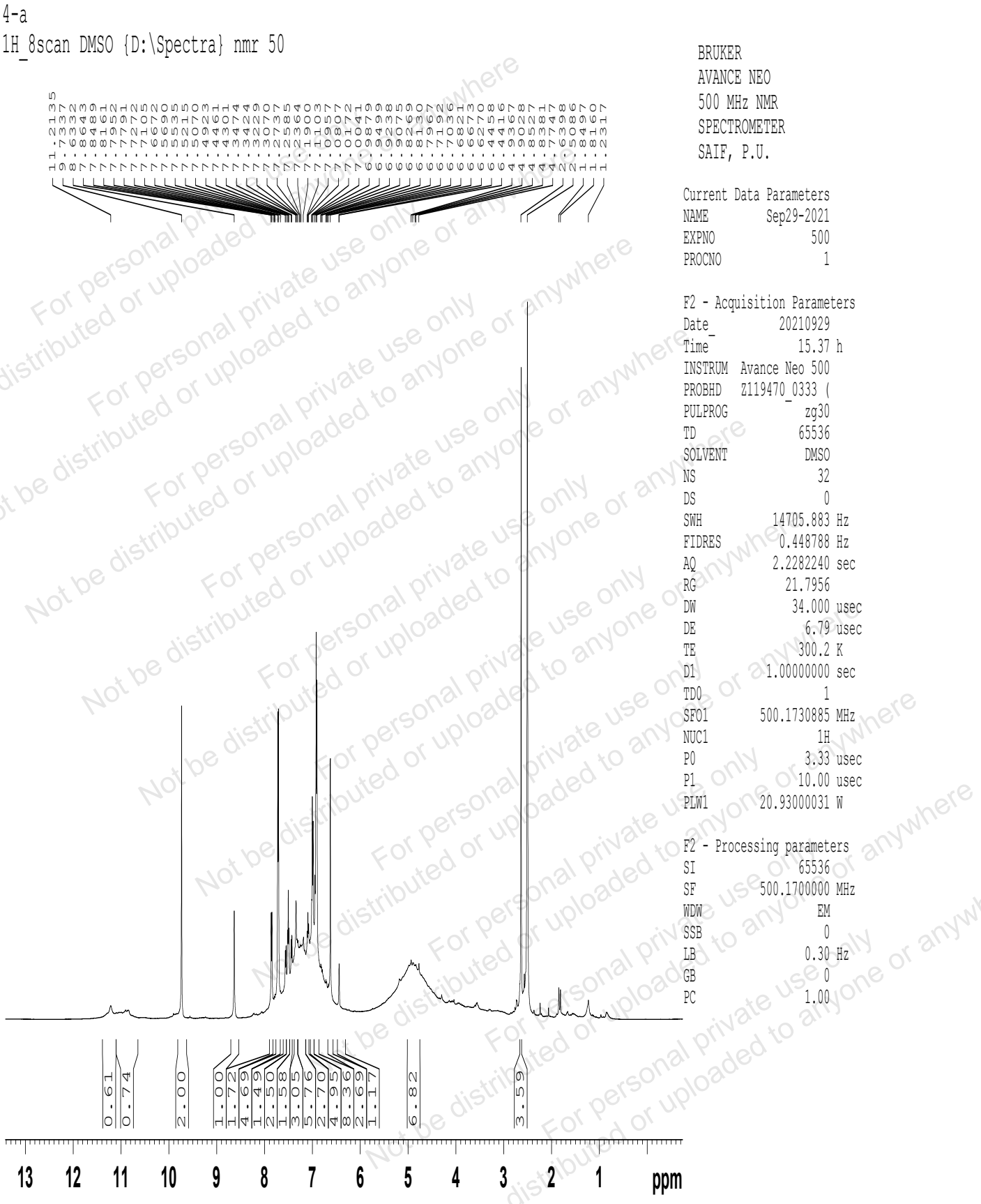




IR Spectra of compound 4b

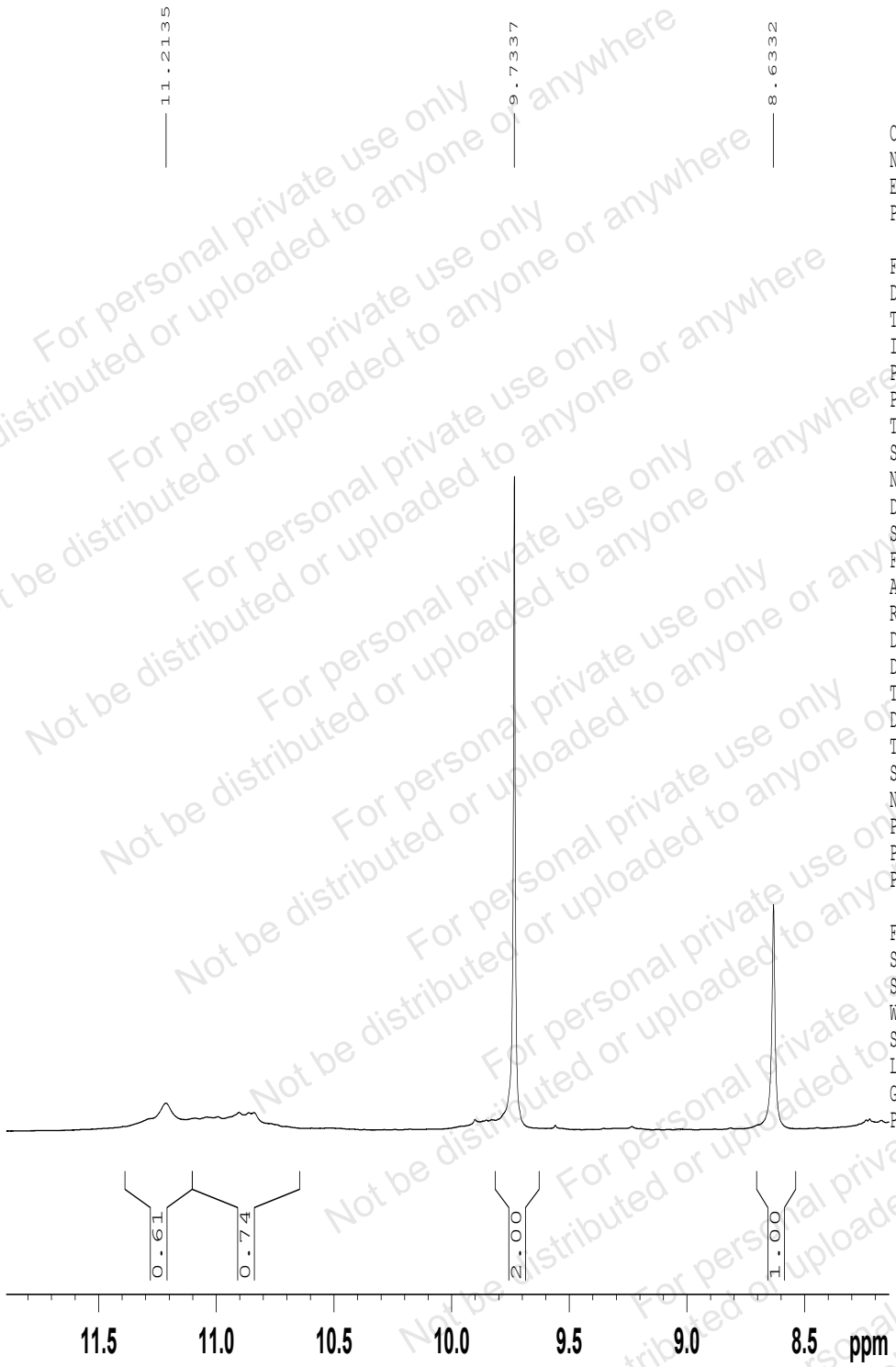


1H-NMR Spectra of compound 4b



4-a

1H_8scan DMSO {D:\Spectra} nmr 50



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

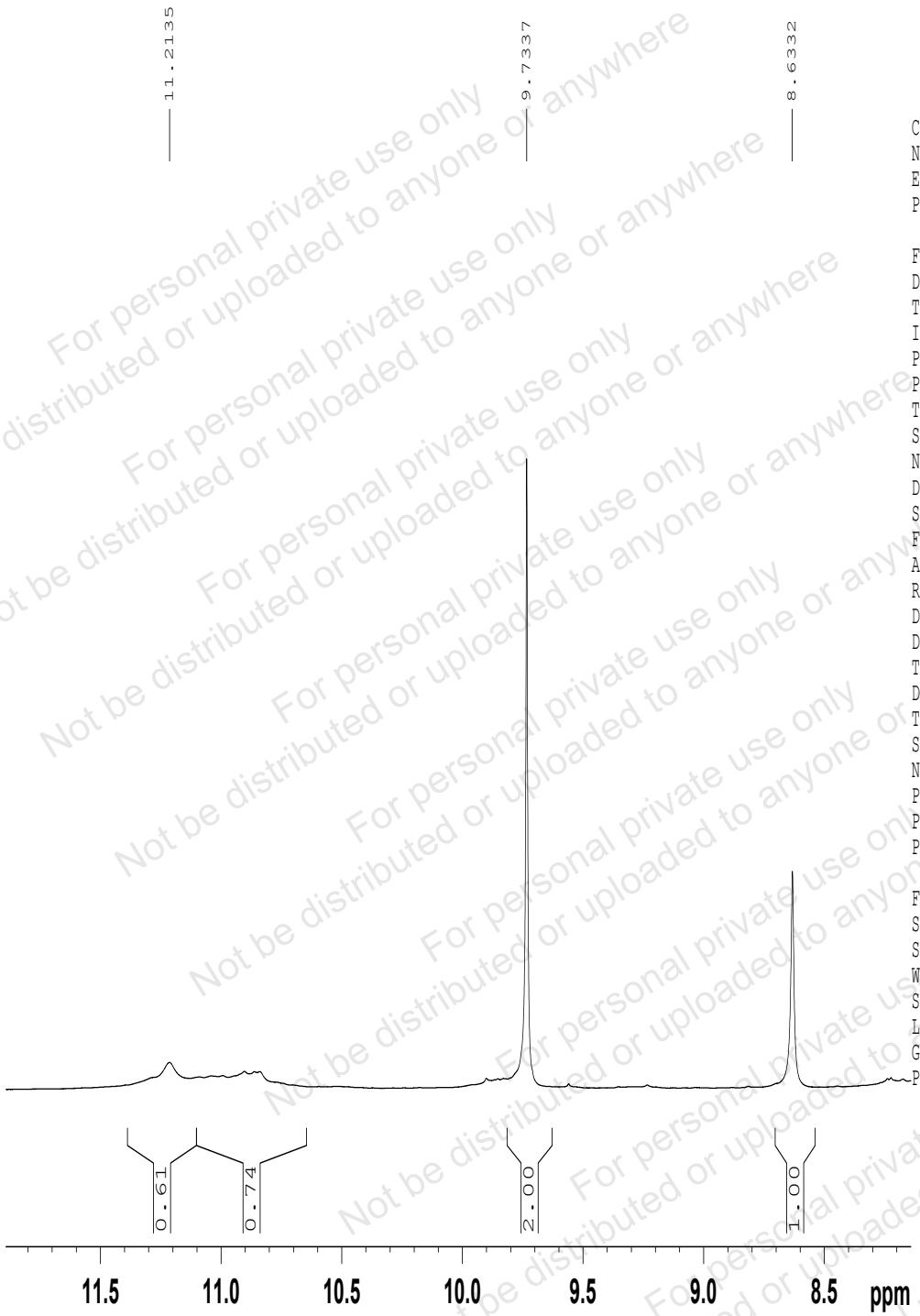
Current Data Parameters
NAME Sep29-2021
EXPNO 500
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.37 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 21.7956
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-a

1H_8scan DMSO {D:\Spectra} nmr 50



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters

NAME	Sep29-2021
EXPNO	500
PROCNO	1

F2 - Acquisition Parameters

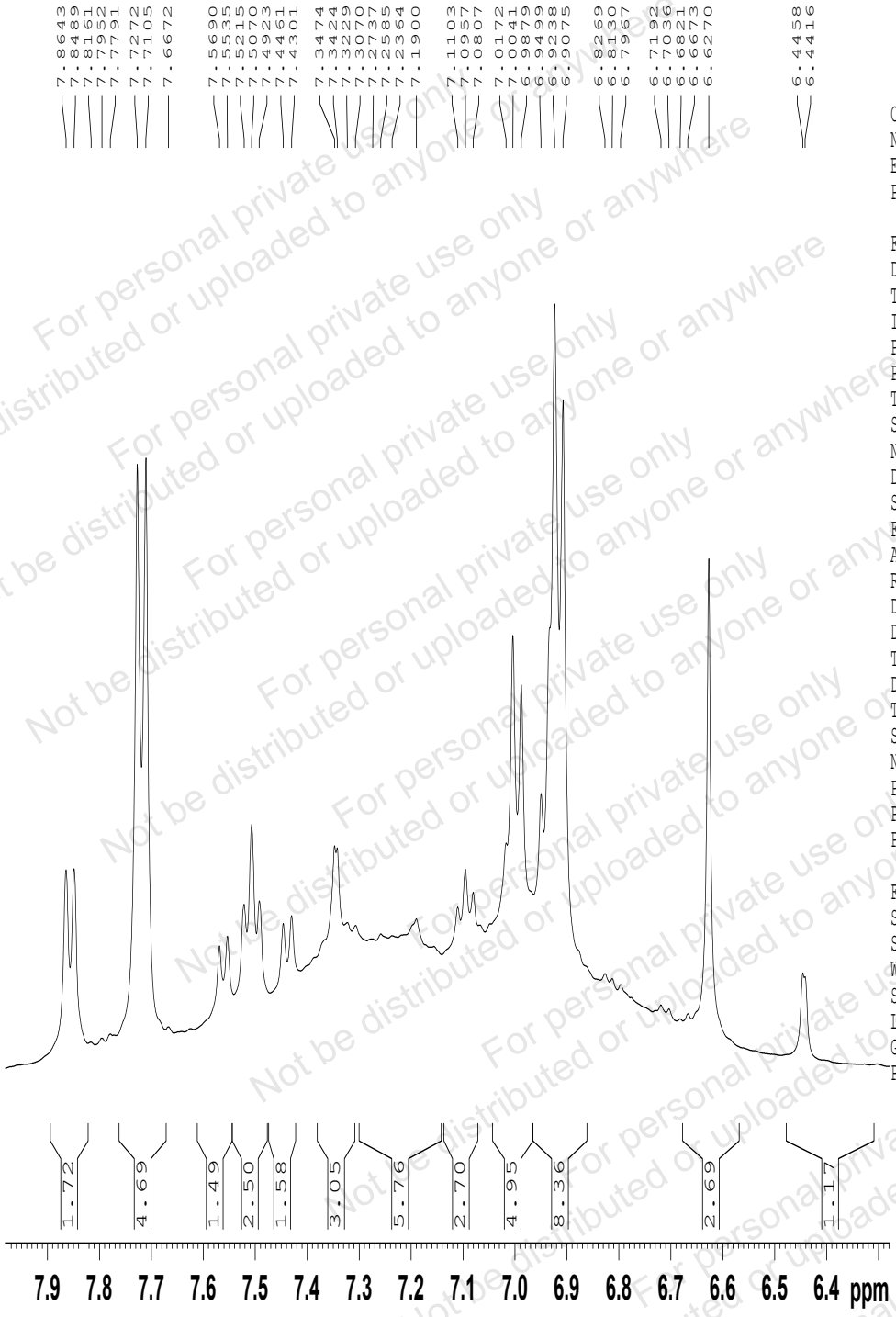
Date_	20210929
Time	15.37 h
INSTRUM	Avance Neo 500
PROBHD	Z119470_0333 (
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	32
DS	0
SWH	14705.883 Hz
FIDRES	0.448788 Hz
AQ	2.2282240 sec
RG	21.7956
DW	34.000 usec
DE	6.79 usec
TE	300.2 K
D1	1.00000000 sec
TD0	1
SFO1	500.1730885 MHz
NUC1	1H
P0	3.33 usec
P1	10.00 usec
PLW1	20.93000031 W

F2 - Processing parameters

SI	65536
SF	500.1700000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

4-a

1H_8scan DMSO {D:\Spectra} nmr 50



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

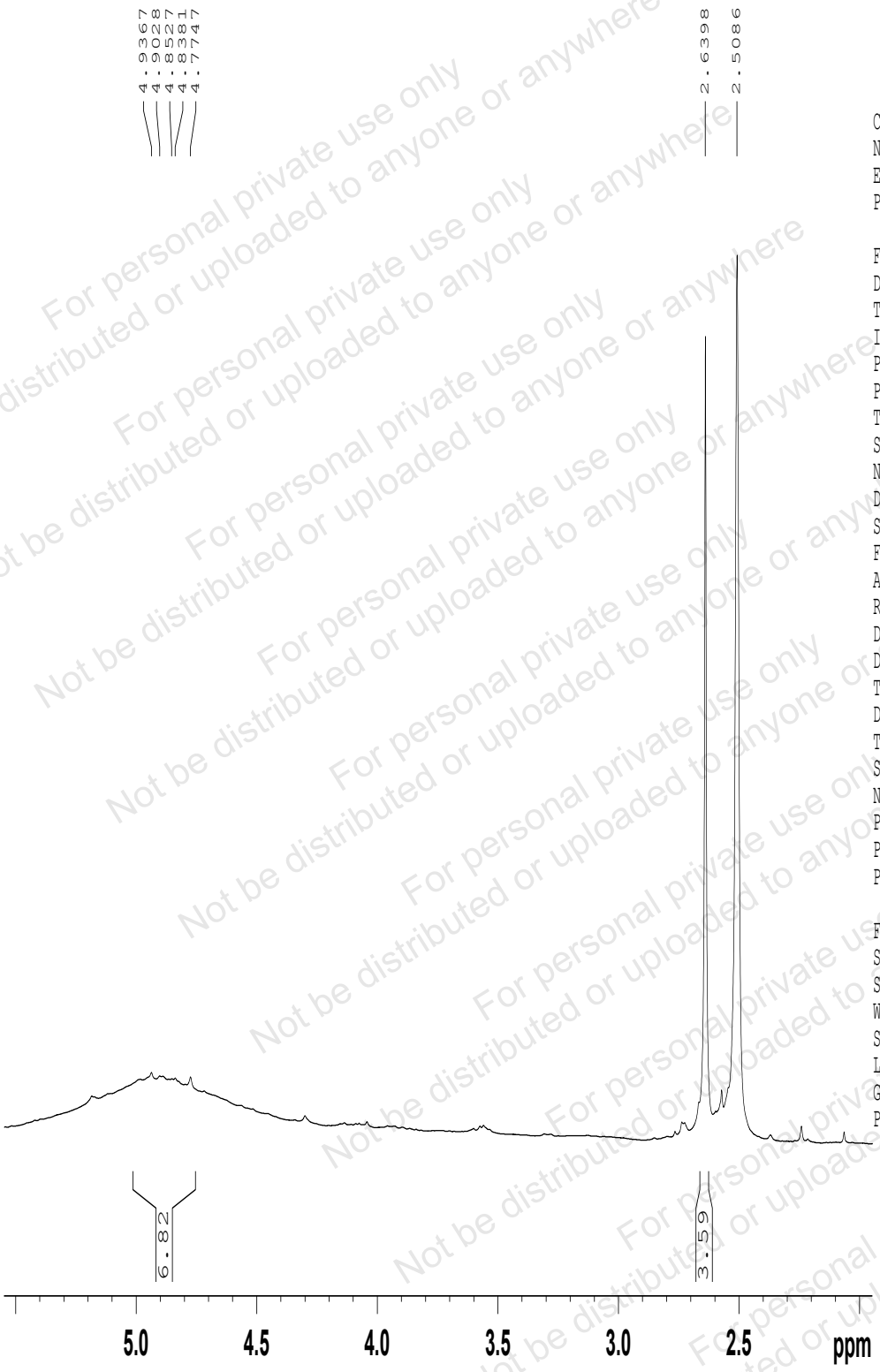
Current Data Parameters
NAME Sep29-2021
EXPNO 500
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.37 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 21.7956
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-a

1H_8scan DMSO {D:\Spectra} nmr 50



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters

NAME	Sep29-2021
EXPNO	500
PROCNO	1

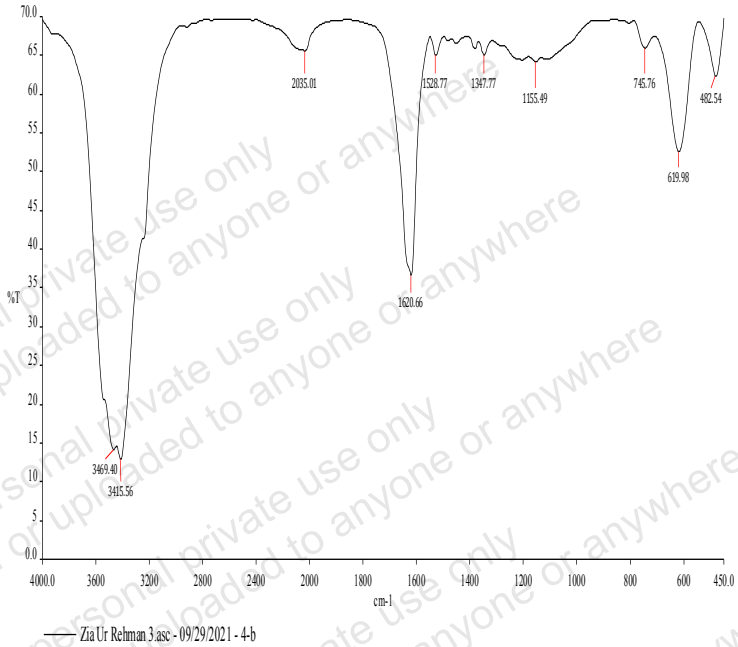
F2 - Acquisition Parameters

Date_	20210929
Time	15.37 h
INSTRUM	Avance Neo 500
PROBHD	Z119470_0333 (
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	32
DS	0
SWH	14705.883 Hz
FIDRES	0.448788 Hz
AQ	2.2282240 sec
RG	21.7956
DW	34.000 usec
DE	6.79 usec
TE	300.2 K
D1	1.00000000 sec
TD0	1
SFO1	500.1730885 MHz
NUC1	1H
P0	3.33 usec
P1	10.00 usec
PLW1	20.93000031 W

F2 - Processing parameters

SI	65536
SF	500.1700000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

IR Spectra of compound 4c



1H-NMR Spectra of compound 4c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BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

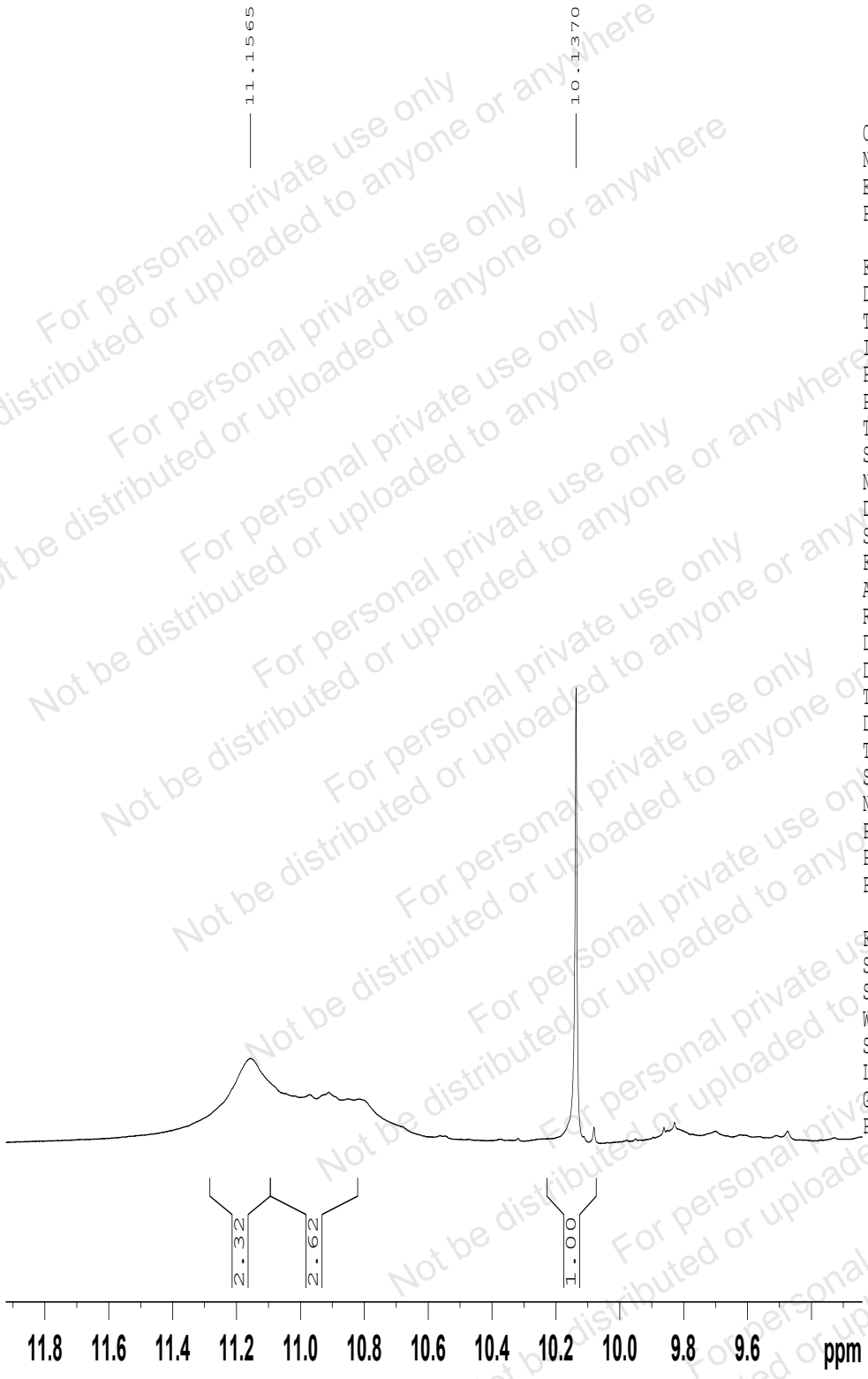
Current Data Parameters
NAME          Sep29-2021
EXPNO                510
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210929
Time_              15.41 h
INSTRUM  Avance Neo 500
PROBHD   Z119470_0333 (
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    32
DS                     0
SWH           14705.883 Hz
FIDRES        0.448788 Hz
AQ            2.2282240 sec
RG              30.2717
DW             34.000 usec
DE              6.79 usec
TE              300.2 K
D1          1.00000000 sec
TD0                    1
SFO1        500.1730885 MHz
NUC1                  1H
P0               3.33 usec
P1              10.00 usec
PLW1        20.93000031 W

F2 - Processing parameters
SI                 65536
SF          500.1700000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

4-b

1H_8scan DMSO {D:\Spectra} nmr 51



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters
NAME Sep29-2021
EXPNO 510
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.41 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 30.2717
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-b

1H_8scan DMSO {D:\Spectra} nmr 51

BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

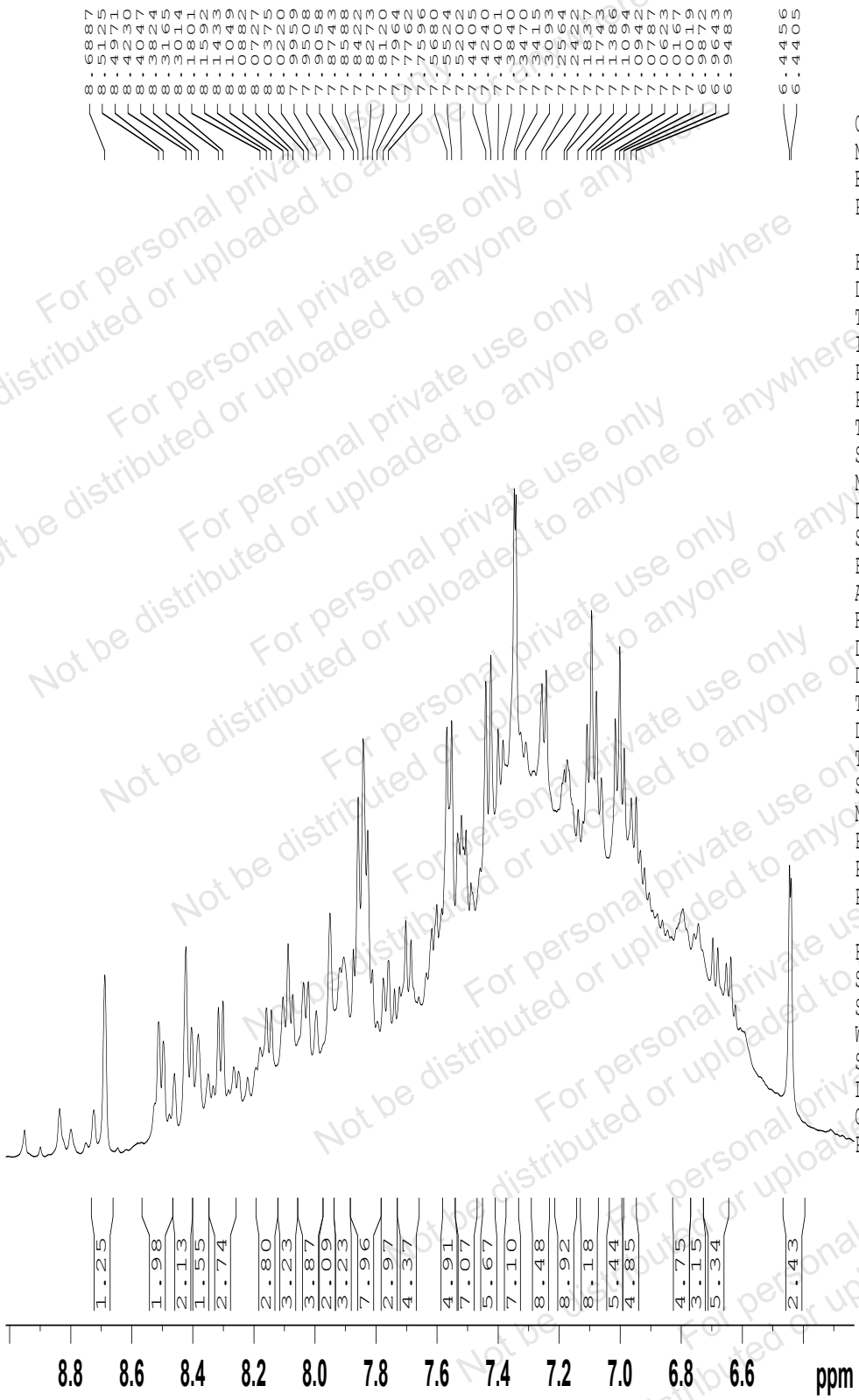
Current Data Parameters
NAME Sep29-2021
EXPNO 510
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.41 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 30.2717
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-b

1H_8scan DMSO {D:\Spectra} nmr 51



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

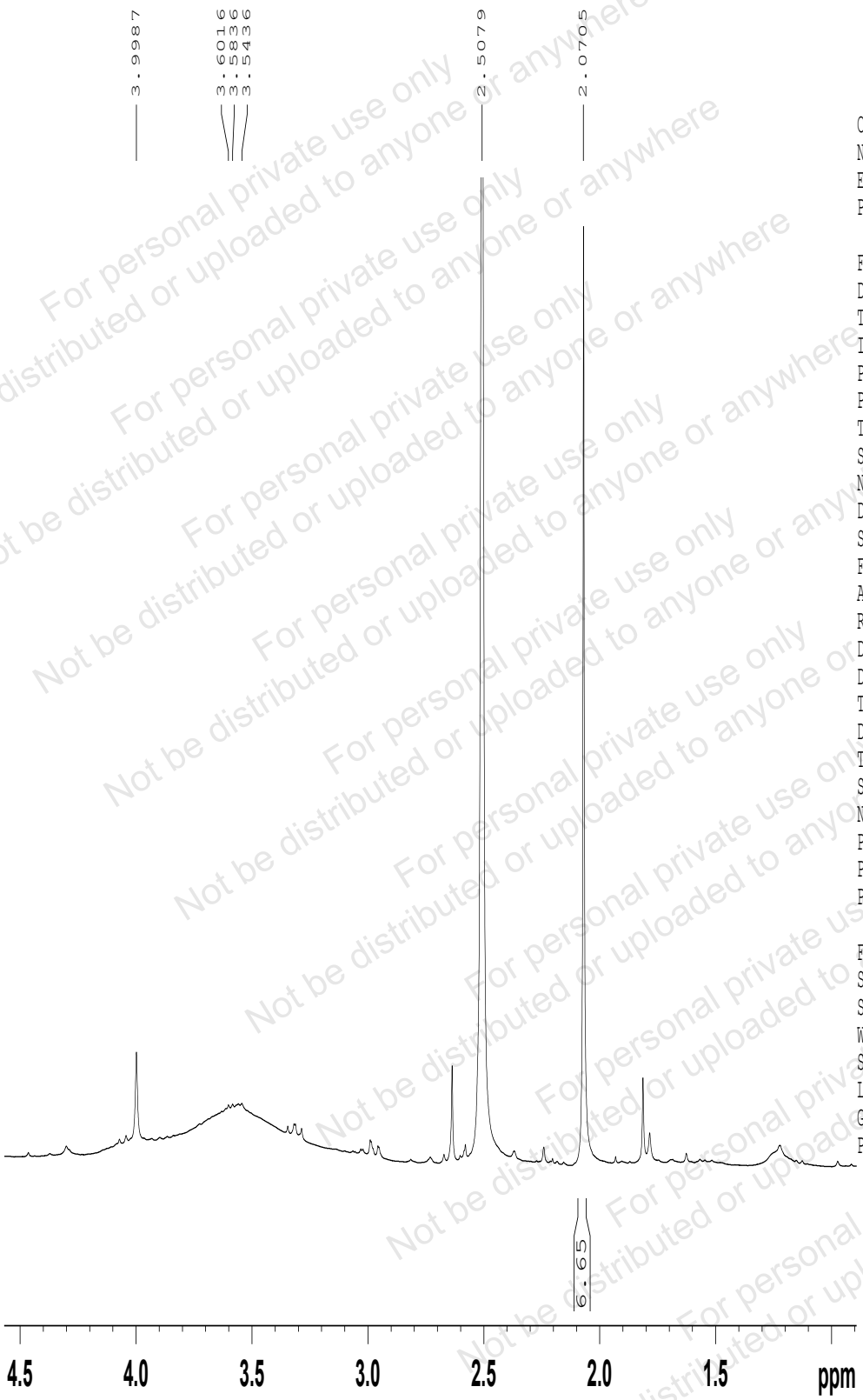
Current Data Parameters
NAME Sep29-2021
EXPNO 510
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.41 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 30.2717
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-b

1H_8scan DMSO {D:\Spectra} nmr 51



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters
NAME Sep29-2021
EXPNO 510
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.41 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 30.2717
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
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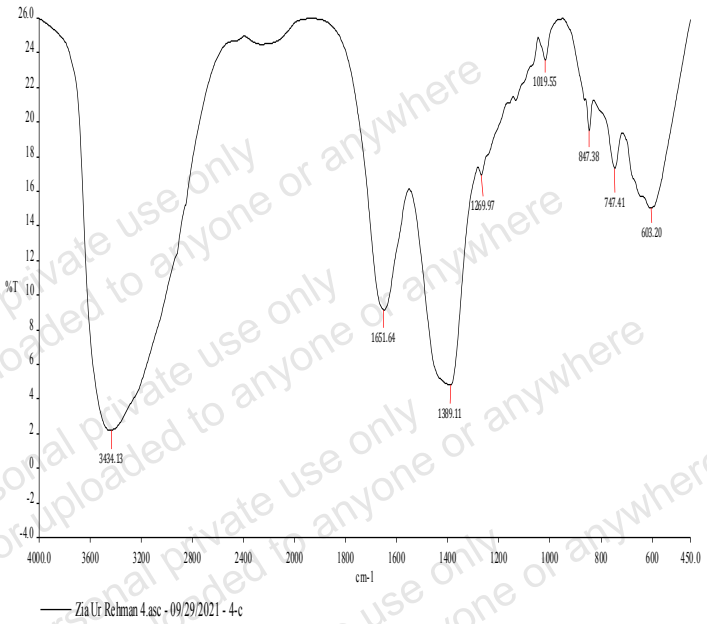
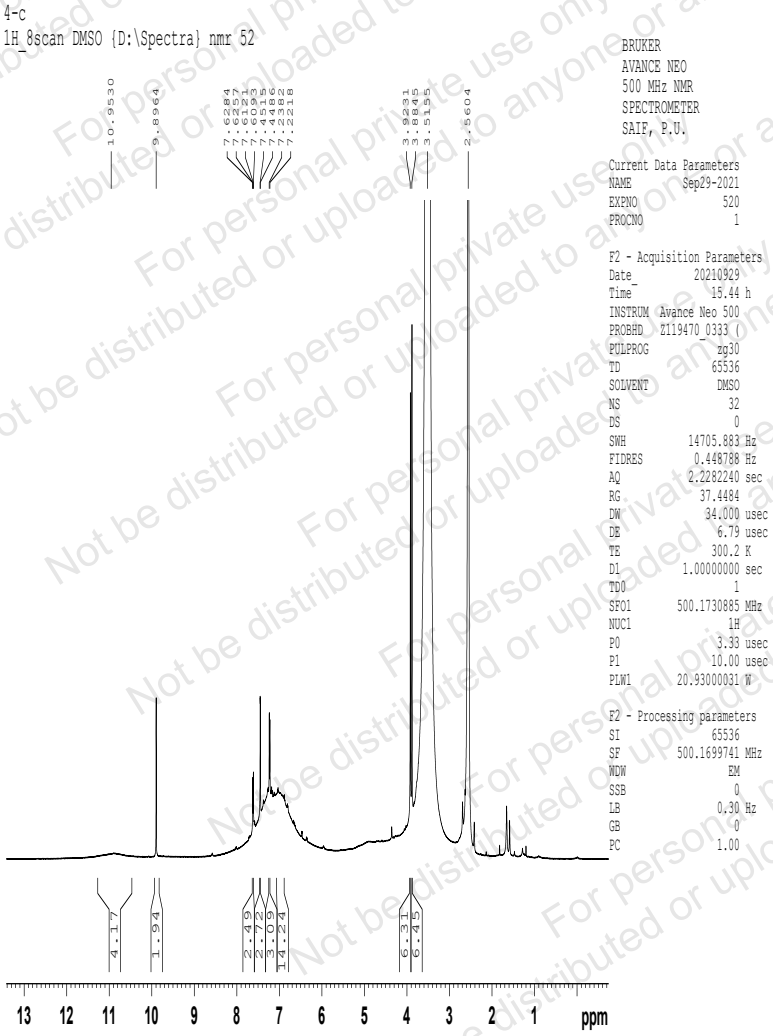
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

IR Spectra of compound 4d

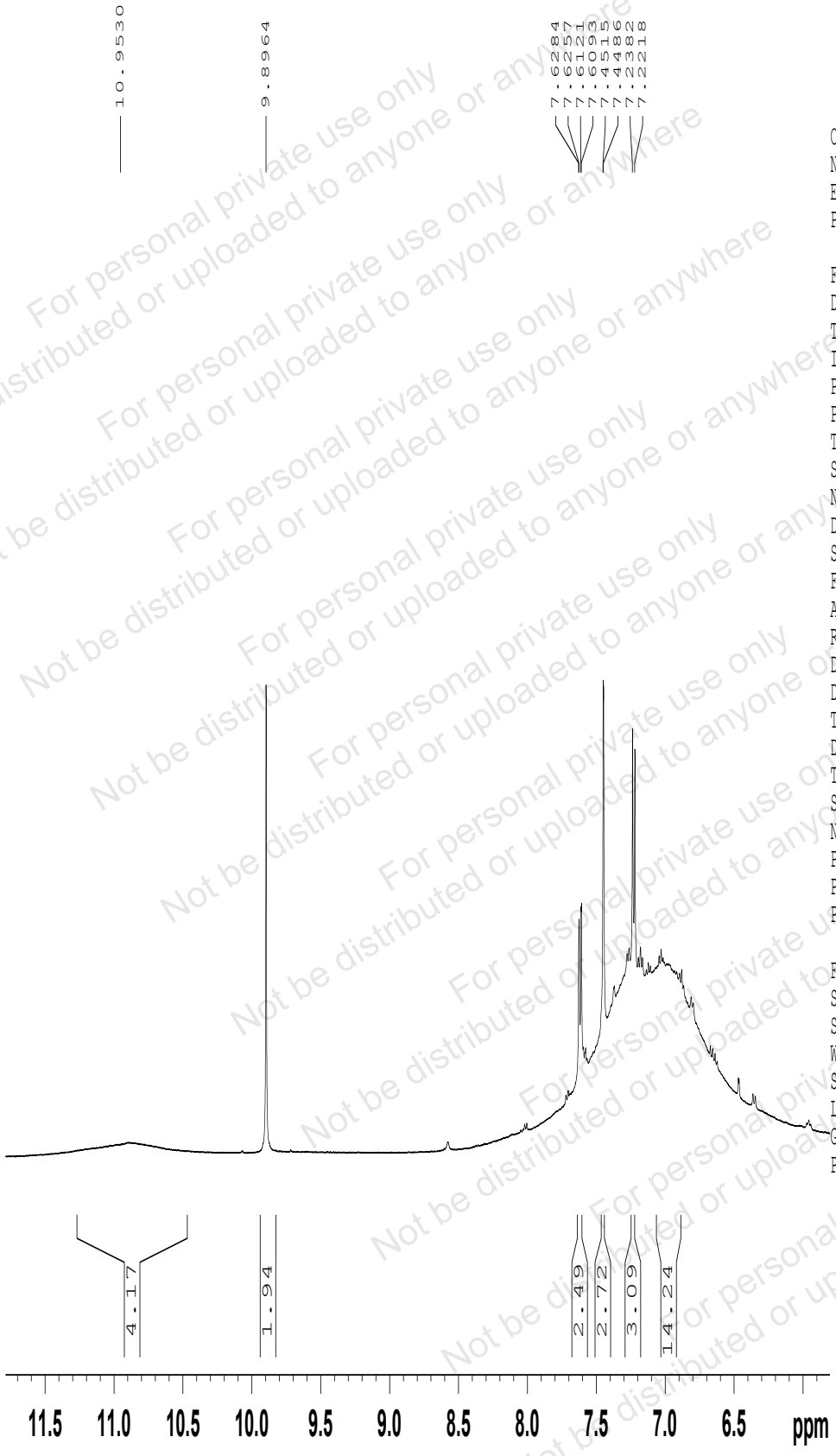


1H-NMR Spectra of compound 4d



4-c

1H_8scan DMSO {D:\Spectra} nmr 52



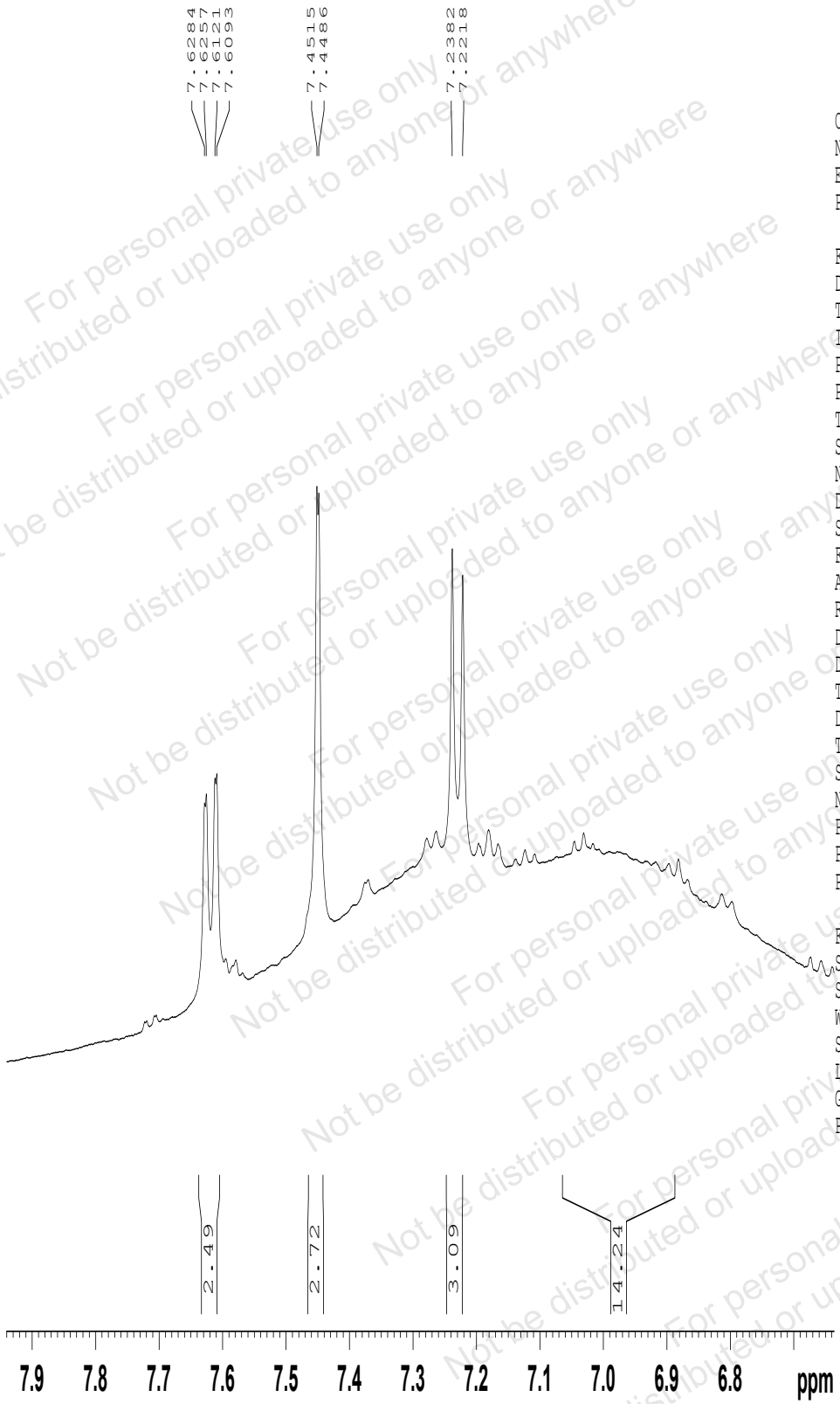
BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters
NAME Sep29-2021
EXPNO 520
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.44 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 37.4484
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1699741 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-c
1H_8scan DMSO {D:\Spectra} nmr 52



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

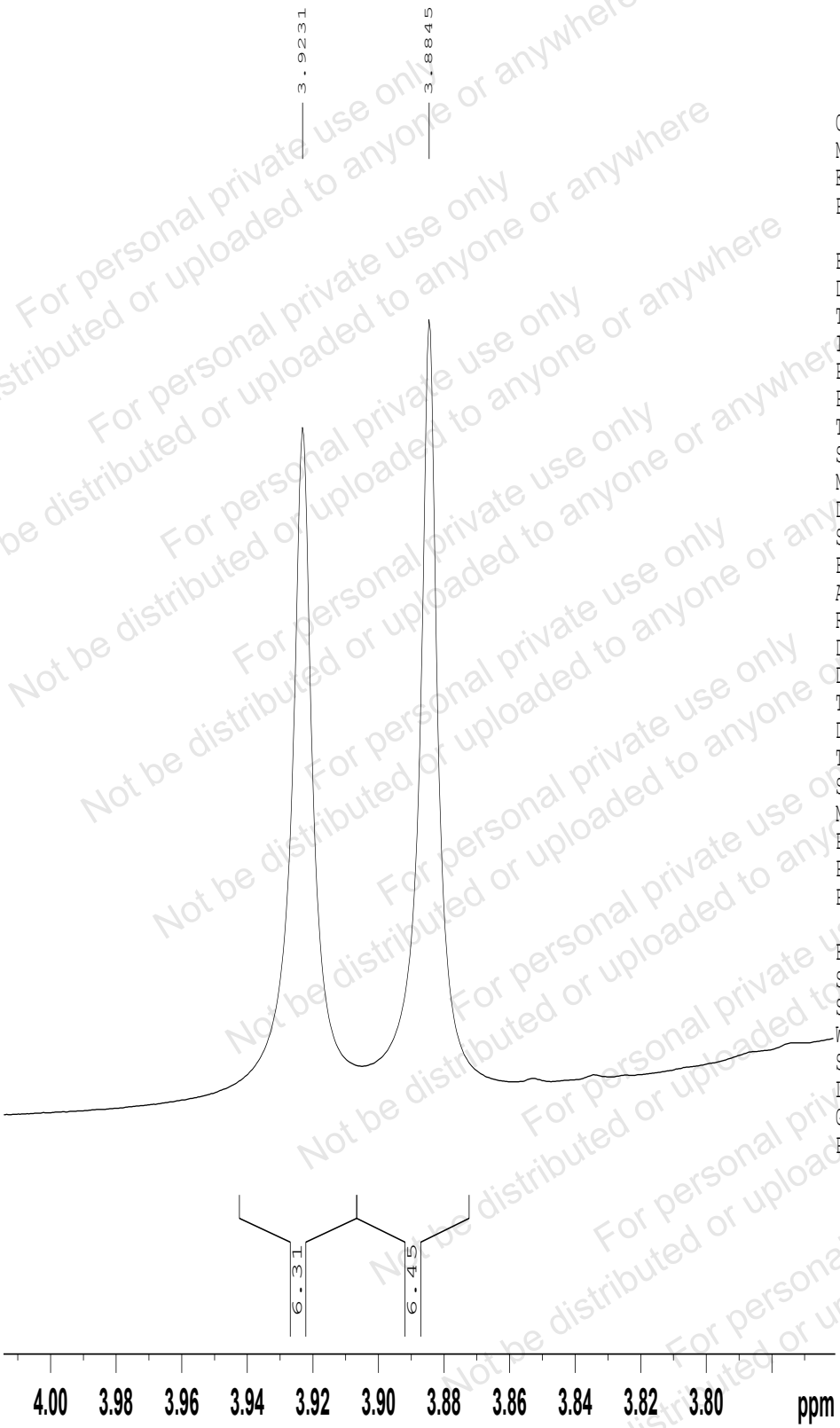
Current Data Parameters
NAME Sep29-2021
EXPNO 520
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210929
Time 15.44 h
INSTRUM Avance Neo 500
PROBHD Z119470_0333 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 0
SWH 14705.883 Hz
FIDRES 0.448788 Hz
AQ 2.2282240 sec
RG 37.4484
DW 34.000 usec
DE 6.79 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1730885 MHz
NUC1 1H
P0 3.33 usec
P1 10.00 usec
PLW1 20.93000031 W

F2 - Processing parameters
SI 65536
SF 500.1699741 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

4-c

1H_8scan DMSO {D:\Spectra} nmr 52



BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIF, P.U.

Current Data Parameters

NAME	Sep29-2021
EXPNO	520
PROCNO	1

F2 - Acquisition Parameters

Date_	20210929
Time	15.44 h
INSTRUM	Avance Neo 500
PROBHD	Z119470_0333 (
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	32
DS	0
SWH	14705.883 Hz
FIDRES	0.448788 Hz
AQ	2.2282240 sec
RG	37.4484
DW	34.000 usec
DE	6.79 usec
TE	300.2 K
D1	1.00000000 sec
TD0	1
SFO1	500.1730885 MHz
NUC1	1H
P0	3.33 usec
P1	10.00 usec
PLW1	20.93000031 W

F2 - Processing parameters

SI	65536
SF	500.1699741 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

REFERENCES

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